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Humble beginnings

Bioreactors have come a long way since their primitive introduction as simple earthenware pots used by our early forefathers in the exploitation of alcoholic fermentation to make beer. Or have they?

The Biopharmaceutical Bioreactor Maze

Eden Biodesign

BEGIN WITH THE END IN MIND

More recent examples of simplistic disposable fermenters demonstrate a back to basics approach; simple by design. But is simplest always best for fermenters? or is it wiser to opt for increased system complexity to cope with the diversity of biopharmaceutical products in the quest for perfect process optimisation? This report examines the types of bioreactors available and their pivotal role in the biopharmaceutical process.

Evolution

There has been considerable recent innovation in the field of bioreactors, from computer aided steel tank designs through to a variety of disposable concepts such as the incarnation of the 'fermenter in a bag'. The sales literature for each bioreactor type often describes a range of advantages over its competitors such as better operational features and the promise of increased productivity - but what is the best way to evaluate these different systems? and how important are the various features in selecting one fermenter over another?

The commercial scale production of biopharmaceuticals still typically uses the traditional approaches of stirred tank, air lift and roller bottle culture. The remit of meeting the intensive market demand requirements of a particular product often relies on bioreactor volumes in the thousands of litres which means coping with the large scale logistics of raw materials handling – literally by the truck load. So why even pause to consider this scale of operation when you're just embarking on developing a new biopharmaceutical process?

Often a fermentation approach is borne out of what's available at the time and usually takes its first uncertain steps under the nurture of the research laboratory. There is often very little regard concerning the scale up beyond a few litres and rarely any consideration of the final capacity required; after all, the first hurdle is purely to make sufficient investigative product. However, paying even a vague attention to where you're aiming to go and the steps required to get there will greatly aid the progression of the upstream bioprocess development. In other words the best approach is to **'begin with the end in mind'.**

Establishment of a robust, reliable and reproducible process

From a regulatory perspective it is important to bear in mind that, for a biopharmaceutical, the process is as important as the final product in demonstrating control and consistency and forms an extensive part of the supporting paper trail. At the very outset, basic fermentation ground rules and assumptions can usually be dealt with as a paper exercise but proof of principle will depend on performing some preliminary bench scale studies and comparative analysis of prospective systems. So should this follow an in depth biophysical approach on expected mass transfer, gaseous exchange, vortexing etc or focus on the type of product that is being derived or expressed? Before extensive tweaking and mathematical modelling, a good starting point is to look at some basic test parameters to examine cell growth and productivity either in static or agitated culture flasks.(see Table 2)

Despite commercially available systems claiming to confer certain advantages, for example in growth and productivity, it should always be remembered that any process is extremely cell line dependant. The cell growth and productivity can change dramatically between wild type to recombinant, host cell to host cell, product to product and different expression vector and gene insert combinations. Although reference examples of similar cell lines and product classes may have been demonstrated to perform well in a particular bioreactor, Murphy's law dictates that your specific cell line will possess a new found individualism that doesn't quite fit the expected profile. A good rule of thumb therefore is to treat every cell line and genetic iteration as being different. At a research level this is not always appreciated as research cell lines are often obtained as gifts from other researchers with resulting indeterminate origins. Even when a cell line is selected from a certified source, often insufficient thought is given to the regulatory acceptability of a particular expression system or ease of cultivation against other potential genetic construction permutations. The only safe approach is to practically examine the characteristics of a cell type or cell expression system with a particular bioreactor to reach a base starting point. If cells are just placed into what ever bioreactor is available then the most optimal approach may be missed, meaning a significant cost impact in

Table 2. Process parameters

- Cell growth
- Productivity
- Maintenance
- Genetic stability
- Long term production

Table 1 Bioreactor formats: points to consider

- Comparative cost of different models and scale
- Capital versus disposable cost
- Scale up issues
- Practicality of operation
- Labour requirement
- Specialist know how requirements
- Regulatory compliance of system and supporting documentation
- Product yield requirements

terms of development time and final production output.

The more complex nature of mammalian cell hosts compared to microbial systems and with respect to the length of fermentation batches makes it even more critical to hit on the correct supportive environment offered from the choice of bioreactors available. A good starting point is to examine the cell characteristics from certified cell bank sources such as the European Collection of Cell Cultures (ECACC), American Tissue Culture Collection (ATCC) and research papers in terms of adherence/suspension and performance traits. The specificity imposed by a particular genetic modification from preliminary research analysis, e.g. basic growth/productivity/maintenance profiles, will lend more refined support in narrowing down a selection of bioreactor types which then may be tested on an actual comparative basis. This may be done in-house or a more comprehensive comparison may be offered by outsourcing to a facility that has a variety of different bioreactor types and the expert know-how to perform efficient multiple comparative studies in a short period of time.

The Bioreactor Universe

Microsystems

A number of small scale bench systems are available which can be anything from classical shake flasks, spinners and roller bottles to more elaborate designs based on small computer controlled banks of miniature stirred tank vessels. At the far end of the inventive spectrum there are now multiwell plate systems and ingenious high throughput cassette bioreactors which can handle multiple cell lines and clonal variations and be programmed to emulate different process recipes in multiple parallel studies. The advantage of the multiwell and especially the cassette approaches is that large scale parameters including stirring can also be miniaturised. Other robotics systems on the market can perform routine maintenance and study analysis of static culture flasks.

Although such microbioreactors are sometimes questioned in terms of their translational scale up applicability they none the less provide a good starting point in the process derivation approach and provide a useful tool to model large scale fundamentals.

The traditional approach of manually using tissue culture flasks and shake flasks is perfectly acceptable but for projects of serious commercial intent this can be labour intensive and slow in contrast to the automated systems. The difference in relative study costs may be a few dollars compared to tens of thousands of dollars but the long term cost benefits and reduced development time for optimisation is vastly in favour of the multiple study approaches and the choice is again to either invest or specialist outsource.

Stainless steel or plastic?

Stirred tank, roller culture, multi tray, bags. Time spent in analysing the capital cost benefits against speed, resource, consumables and assumptions matched with early research analysis on, for example, the growth and productivity characteristics in line with different fermenter formats will help create a firm foundation for pilot scale upstream development. Even at this stage a narrowed down selection of bioreactors can be compared.

Stirred tank

The classic stainless steel stirred tank fermenter has proven robustness in supporting a variety of microbial, yeast and mammalian derived products including, in the case of mammalian cell lines, free suspension and microcarrier suspension for adherent cells.

The advantages of this system are that it is a tried and tested technology and that the parameters are easily configurable. The disadvantages are that the preparation of the unit and clean-down are very labour intensive and scalability is uncertain, usually requiring further development at each successive jump in scale. The luxury of running multiple units for quicker and statistical process development can be expensive in capital but performing thorough studies in singular batches will extend development phases significantly. Different agitation approaches have been adopted, the most notable being high aspect ratio air lift. The advancement in aseptic connectors and tube welders greatly aid aseptic transfer and diminish the risk of contamination.

Roller bottles

Roller bottles are another historic bioreactor format, and one of the first incarnations of a disposable fermenter technology. Ideal for adherent cell and especially primary cell lines, their design has recently been augmented by inserting cell support matrices to increase cell growth numbers. The simplicity and modularity of roller bottles makes them highly suited to fast track optimisation as multiple studies can be done in parallel. Manual operation can be performed up to one to two hundred bottles, however beyond this operator repetitive strain issues are a real concern. Automated robotics systems increase the scale of operations, but evens these are still only to the limit of several hundred bottles. In fact as any system augments in size the extended process time can be a significant factor in being able to fit everything into a working day before resorting to shift patterns. Housing roller bottles rigs requires a large facility footprint which is another important consideration. A significant advantage of roller bottles is that due to their modular nature they exhibit linear scale up and therefore a greater certainty of success. Expanded surface roller bottles further support higher density monolayers.

Bag fermenters

By adding platform agitation, the successful principle of plastic bioprocess containers (BPCs) has been developed into bag based bioreactors. The simple, disposable nature of these units allow them easy adoption into a wide variety of facilities for a range of uses. Large volumetric sizes have recently been introduced which have added good scale up potential. However, similarly to stirred tank, there is no guarantee of direct scale up. Flat bed bag systems exhibit low shear and reduced foaming which is normally associated with conventionally agitated systems.

Bag based fermenter technology has recently taken a further step forward by the incorporation of an impeller to offer a truly disposable stirred tank option. This greatly enhances the applicability of this format in the easy accommodation of a range of volumetric sizes in a range of institutions and facilities for a reduced capital outlay. The advent of disposable probes has also complemented the arrangement of bag systems in creating a fully disposable 'plug and play' bioreactor format. This greatly facilitates set up through not having to sterilise or perform cleandown validation. The long term continual disposable use of the systems, including the probes, may not currently be a cost effective option for research establishments but they present an option worthy of consideration for commercial product development.

Others

Other small to medium scale bioreactor formats include stacked trays and double sided mutli plates packed into a cube configuration. Whilst these can be pushed to the limit for small scale production the other use is for cell expansion in the generation of a seed pool. Again the advantage is linear scale up which is particularly useful for this phase of production. Particular attention has been paid to the plastics used and applied surface treatments to support better cell growth with these systems.

Small permeable and packed bed perfusion systems exist for small scale applications and hollow fibre units have grown in size in support the traditional small to medium scale monoclonal production in continuous culture. Of note, cell growth through continuous culture is less favourable than batch production from a regulatory perspective due to the difficulty in demonstrating consistency both within and between batches.

Control systems

This review has principally focused on the different bioreactor vessel designs but obviously the control systems used to operate these are a key element in ensuring consistent and defined operation. Most vessels are connected to mass flow units for gas and fluid exchange, operated via solid state process logic control interfaces. For research and development these can be linked to computer analysis software to monitor the status of the fermentation and allow for programmed feeds etc. In a manufacturing environment this supervisory control and data acquisition aspect is required to meet CFR 21 part 11 compliance and primary importance is placed on data recording and alarm state monitoring. Control systems have similar architectures in most respects and some can run different vessel formats through coordinating the signals received from the various probes, actuators and feedback loops.

There is a regulatory move towards greater adoption of Process Analytical Technology (PAT) which places a greater emphasis on controlled on line monitoring. Already there are an increasing number of metabolic monitoring probes but the long term goal is online product monitoring. If this becomes available it will provide the ultimate fine tuneable optimisation for control of a fermenter process.

Production scale

The expansion to provide sufficient product to meet market demand for a successful biopharmaceutical requires full bioengineering support and is usually through stirred or air lift high volumetric units. The increasing ability

Bioreactor	Advantages	Disadvantages
Stirred tank	• Robust design • Support of large volumes • Small to large scale production	• Non linear scale up • Sterilisation and cleandown validation • High maintenance • High capital outlay
Roller bottles	• Simple • Minimal control • Disposable • Modular • Linear scale up • Small to semi-large production scale	• Robotics required at large scale (capital cost) • Labour intensive at small scale • Time consuming • Large footprint of roller rigs at larger scale • Large Incubator space required
Bag Fermenter	• Simple • Disposable • Small to semi-large production scale	• Large footprint at larger scale
Multitray	• Disposable • Small to medium scale production • Suited to cell expansion	• Labour intensive • Incubator space required for larger units
Multiplate	• Disposable • Small to medium scale production	• Difficult to assess monolayer integrity
Micro well	• Versatile • Limited multivariable studies	• Limited throughput
Micro cassette	• Versatile • Rapid High throughput analysis • Extensive multivariable studies	• Capital cost of associated robotics

to optimise productivity means that market demand can potentially be met using smaller bioreactor sizes in the future. In practice however, the inevitable drive to reach market quickly means that maximal process optimisation is often not achieved. Outsourcing this part of development to specialist companies able to do this quickly and efficiently will reduce risk of programme delays and prove extremely cost effective in the long run.

Decisions, Decisions

The choice of bioreactors is becoming more and more expansive with an ever growing options list. So which is best? In short, it depends on your product.

The most important decision driver is matching your cell expression system to the best supportive format and not the other way round. A diligent paper exercise and directed studies will create a firm foundation platform in determination of a reproducible, robust and reliable process. Then all you need to do is worry about the purification!

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